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(54) Title: HUMAN TUMOR CELLS IMPLANTED IN NON-HUMAN ANIMALS

(57) Abstract

A non-human animal model is provided with human cancer cells supported on a support including a biological response modifier, with the animal preferably being immunodeficient with respect to its own immune system. The animal model can also be provided with a human immune system. The model can be used for testing cancer treatment for primary human cancer cells.

* See back of page

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HUMAN TUMOR CELLS IMPLANTED IN NON-HUMAN ANIMALS

This invention relates to tumorigenesis and more particularly to maintaining and testing human cancer cells in non-human animals.

While some tumor cells can be readily grown in culture, they do not, in the environment of the tissue culture vessel, maintain the identical properties of the primary tumor. In fact, they may frequently represent a subtype or selected subpopulation of the primary tumor. Although the property of continuous, and non-contact inhibited growth may persist, numerous other characteristics, including the genes expressed, the nature and quantity of the markers expressed on the cell surface, and the response to hormones, biological response modifiers and other growth factors may be highly variable, and different from the primary tumor of origin.

There exist certain animal models for the maintenance and propagation of tumor cells. In mice, for example, tumor cell models exist, where injection or transplantation of cells will result in tumor development. However, such tumor cells have undergone significant changes from the original primary tumor cells, in becoming adapted to propagation in animals. In addition, it is not a

certain or straight forward process to generate such transplantable tumor cells lines, since only a fraction of primary tumor cells will have the ability to be adapted in this way.

The difficulties are even greater if one attempts to cross species barriers in such transplantable tumor cells. Human tumor cells, for example, will normally succumb to the immune system of mice. One way that has been developed to overcome this, in certain circumstances for certain human tumors, is use of the 'nude' mouse strain (S.P.Flanagan, Genet. Res. Camb. (1966), 8, 295-309. ""Nude," a new hairless gene with pleiotropic effects in the mouse."). In these mice, defects in the immune system allow the survival of certain foreign cells injected or transplanted into the animal. However, even here only a variable fraction of primary human tumors can be successfully propagated.

Thus, there is a need for effective maintenance and propagation of primary human tumor cells in an animal model.

In accordance with an aspect of the present invention, human cancer cells are administered to, and maintained in, a non-human animal wherein the non-human animal is immunodeficient.

In accordance with a preferred embodiment of such aspect of the present invention, the human cancer cells are maintained in the non-human animal which is immunodeficient with respect to its own immune system, on a biocompatible support which may include a growth factor or factors for inducing angiogenesis to the implant, and which may be required for the growth of the cancer cells. (Biological Response Modifiers, or BRMs.)

In accordance with another aspect of the present invention, human cancer cells are maintained in a non-human animal on a biocompatible support which may include such angiogenic or growth factors (BRM's). The non-human animal may or may not be an immunodeficient animal.

In accordance with a further aspect of the present invention, the non-human animal which includes human cancer cells, in accordance with an embodiment as hereinabove described, is employed as an animal model for studying the effect of a cancer treatment or therapy.

In accordance with a further embodiment of the present invention, a non-human animal which includes human cancer cells in accordance with an embodiment as hereinabove described, further includes a human immune system, in particular the animal is provided with human hematopoietic cells which create a human immune system in the non-human animal.

The non-human animal is preferably a rodent.

As hereinabove indicated, the human cancer cells as preferably maintained in a non-human animal on an implanted biocompatible support which may include BRM's, a factor or factors to induce angiogenesis and cell growth. This implant is sometimes hereinafter referred to as a neovascularization device.

The biocompatible support may be a biodegradable support (sometimes referred to herein as an absorbable support) or a nonbiodegradable support (sometimes referred to herein as a nonabsorbable support), preferably a non-biodegradable support.

The neovascularization device or devices can also include in addition to a biological response modifier an extracellular matrix component or components. The support may be formed from an

extracellular protein or the extra-cellular matrix component may be added to the support. For example, the support may be formed from collagen which is an extracellular protein or an extracellular component may be added to the support.

A biological response modifier (BRM) is a biochemical agent that directly or indirectly induces a change in gene expression at the cellular level. Growth hormones and their chimeric derivatives serve as examples of a direct BRM; initiating a biological response ultimately directed to the nucleus and mediated by a specific high affinity receptor. Hydrolases (plasminogen activators, collagenases, heparinase) serve as an example of an indirect BRM; initiating a biological response by enzymatically activating or releasing latent, stored or zymogen precursors of direct BRMs. The BRM may stimulate or facilitate vascular growth from a tissue or organ, and may be required for growth of cells on the support.

Biological response modifiers used on the support can be angiogenic growth factors such as HBGF-I, HBGF-II, platelet-derived growth factor (PDGF), macrophage-derived growth factor (MDGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), hypothalamus-derived growth factor (HDGF), retina-derived growth factor (RDGF), and mixtures thereof. A preferred embodiment of the invention for inducing angiogenesis uses HBGF-I. Desirable hydrolases include a member selected for the group consisting of heparinase, collagenase, plasmin, a plasminogen activator, thrombin, heparinase, and mixtures thereof. Hormones such as the growth factors are particularly desirable as

biological response modifiers. Hormones specifically elicit cell growth and differentiation.

The neovascularization product can also include an extracellular matrix component, which may be the material from which the support is formed or may be added to the support. Although applicant does not intend to be bound by theoretical reasoning, it is believed that the inclusion of the extracellular matrix component may, among other functions, function to hold and to protect the BRM which is included in the product; i.e., provides for adsorption and adherence of the BRM to the support and possibly protects against degradation of the BRM. In addition, the extracellular matrix component may function to absorb cells which participate in the neovascularization or tumor cells added to the device. One or more extracellular matrix components, which may be used alone or in combination, include collagens, laminins, fibronectins, gelatins, glycosaminoglycans, glycoproteins, proteoglycans, and mixtures thereof. The most desirable extracellular matrix components include gelatins and collagens. A preferred embodiment uses collagen Type IV. Collagen Type IV is desirable because it provides desirable adsorption characteristics for the biological response modifier.

The support may be either an absorbable (biodegradable) support or non-absorbable (non-biodegradable) support and the support can be formed from a extracellular matrix component. The preferred support is a non-absorbable support which includes at least one extracellular matrix component to which at least one BRM may be absorbed. Examples of absorbable supports include members of the group consisting of collagen Type I, known commercially by

the trade name "Gelfoam", laminin, fibronectins, gelatin, glycosaminoglycan, glycolipids, proteolipids, mucopolysaccharides and mixtures thereof. Examples of non-absorbable matrices include members of the group consisting of nylon, rayon, dacron, polypropylene, polyethylene, polyurethane, expanded polytetrafluorethylene (PTFE), cross-linked collage Type IV, and mixtures thereof.

The support is one which is biocompatible with the host in which it is to be implanted and has the rigidity and strength to support growth of the added cells.

Expanded polytetrafluorethylene (PTFE) has been found to be a particularly suitable non-absorbable support. This support provides the following benefits. PTFE has a general lack of an inflammatory response which is the basis for the current acceptance of PTFE in the surgical community. PTFE can be coated conveniently with extracellular matrix components which can absorb a biological response modifier. Biologically active HBGF-I and HBGF-2 can be immobilized to collagen-coated PTFE by previously established methods. PTFE polymers are routinely engineered to various specifications to meet a multitude of required configurations.

Non-woven multifilament angel-hair fibers of expanded PTFE are commercially available from W. L. Gore and Associates, Inc., Flagstaff, Arizona and are particularly suitable as a support. These fibers allow sufficient organized area for infiltrating cells to be exposed to the environment of the host. This permits the free exchange of nutrients and toxic waste to occur while neovascularization processes occur.

Supports can be provided for use in this invention in any desired shape and size. Desirable shapes for a support can be a strip, a sponge, or a tube. Supports are desirably capable of being secured within an organism. Suitable means for securing a support can include a staple, biocompatible glue, or other surgical procedures such as suturing or tying the support to a tissue.

A desirable support is obtained by filing a tube or sleeve of expanded PTFE with expanded PTFE fibers or "angel hair".

For example, HBGF-I is known to bind to components of the extracellular matrix and components of the extracellular matrix can either be immobilized on solid supports or denatured and formed into an insoluble matrix. HBGF-I, adsorbed to either denatured collagen I sponges, or to fibers of expanded PTFE coated with either fibroneotin, collagen I or collagen IV, or to combinations of denatured sponges wrapped with PTFE fibers, promotes angiogenesis in both the normal and immune compromised (BNX) mouse at concentrations which are consistent with the growth, see below factor's activity as a cellular mitogen in vitro.

The most effective concentrations for a biological response modifiers can be a concentration that elicits a growth response from the target cells, but is not toxic to those cells. Effective or therapeutic concentrations of angiogenic growth factors are between about 1 to 100 nanograms per cubic millimeter of a support. A support for this calculation includes both the absorbable support and the non-absorbable support.

In accordance with an aspect of the present invention, a device of the type hereinabove described is seeded with human cancer cells prior or subsequent to implantation of the device in a non-human animal.

The non-human animals which are provided with the human cancer cells are preferably immunodeficient non-human animals; in particular immunodeficient mice and rats. Such immunodeficient mice are known in the art and may for example be SCID mice or mice of the beige/nu/Xid, (BNX) genotype, [McCune S.M., Namikawa R., Kaneshima H., Shultz L. D., Liberman M., Weissman IL: The "SCIDHu Mouse: Murine Model for the Analysis of Human Hematolymphoid Differentiation and Function" Science 241: 1632-1639 (1988). Mosier D.E., Gulizia R.J., Baird S.M., Wilson D.B.: "Transfer of a Functional Immune System to mice with Severe Combined Immunodeficiency". Nature 335: 256-259 (1988). Kamel-Reid, S. and Dick, J.E.: Engraftment of Immune-Deficient mice with Human Hematopoietic stem cells, Science 242: 1708-1709 (1988)] and which have little immune function left to interfere with the growth of foreign cells. These mice have been extensively used for human tumor xenografts (Isaiah J. Fidler, Cancer and Metastasis Review, 5:29-49 (1986), "Rationale and methods for the use of nude mice to study the biology and therapy of human cancer metastasis"; Seiji Naito, et al., Clin. Expl. Metastasis, vol. 5, #2, 135-146, (1987), "Growth and metastatic behavior of human tumor cells implanted into nude and beige nude mice,"; Jurgen Mattern, et al., Cancer and Metastasis Reviews, 7:263-284, (1988), "Human tumor xenografts as model for drug testing,"; Andrew Pawlowski and Peter J. Lea, in Skin Tumore: Experimental and Clinical Aspects, edited by Claudio J. Conti, et al., Raven

Press, New York, (1989), "Human Melanoma Xenografts".).

Although in a preferred embodiment, the human cancer cells are placed on a neovascularization device of the type described in an immunodeficient non-human animal, it is possible within the spirit and scope of the invention to use a non-immunodeficient non-human animal.

In accordance with another embodiment of the invention, the immune deficient non-human animal, in particular mice, are provided with other human cells in addition to the primary human cancer cells. First, these may be precursor cells that will provide the animal with a human immune system. This kind of device allows testing of cellular and biological procedures for arresting tumor cell growth or for tumor destruction. In particular, examples are adoptive immunotherapy procedures such as LAK therapy (Rosenberg,S.A., J.N.C.I. 75, 595-603, 1985; Mule, J.J., et al., Science 225, 1487-9, 1984) or TIL cell therapy (Rosenberg, S.A., et al., New Engl.J.Med. 319, 1676-80; Rosenberg, S,A., et al., Science 223, 1318-21, 1986.), or any agents capable, on administration, of stimulating the newly generated immune system in the mouse to arrest the growth of, or kill the tumor cells in, the implant. This system is used in an autologous fashion e.g., the immune system of a patient is duplicated in the immune deficient mice, while tumor cells are implanted via the implant, and then the particular therapies applied.

Second, other cells in addition to the tumor cells may be applied in the implant. These could be any of a wide variety of cells known to be able to grow on such an implant system, and may include, but

not be limited to, fibroblasts, smooth muscle cells, endothelial cells, epithelial cells, hepatocytes, mesothelial cells. Such cells may function to support tumor cell growth by a variety of mechanisms eg by secretion of particular growth factors, angiogenic factors, extracellular matrix components, or other factors, all of which may constitute the appropriate micro environment for maintenance of the properties and growth of the primary tumor cells. Additionally, cells may be added and screened for the property of production of agents, or other properties of inhibition of the growth of tumor cells which usually grow well on the support without additional cells.

The human cancer cells which are used in the present invention are so-called primary human cancer cells (i.e., cells taken directly from a patient's tumor and maintained in vitro only for the shortest possible time). The cancer cells may include, but are not limited to the following types; breast, lung, ovarian, melanoma, colorectal, renal.

Thus, in accordance with an aspect of the present invention, human cancer cells are placed in a non-human animal in an in vivo physiological environment that maintains the appropriate properties and characteristics of the original human primary cancer cells. In this manner, such human cancer cells may be tested and/or studied and/or treated in a non-human animal model under in vivo conditions which do not alter the characteristics of the cancer cells.

Thus, the present invention can provide a non-human animal model system for testing, studying, manipulating and predicting behavior of tumor cells

and developing treatments for primary human tumor cell lines from a variety of different cancers.

In accordance with the present invention, the properties of cells of an individual human patient may be developed in the animal model and be used to predict the growth and behavior of such cells in the patient.

In addition, a patient's tumor cells may be established in a number of different non-human animals and such animals may be subjected to a variety of therapeutic procedures (singly or in combination; chemical and/or physical) to thereby determine the best course of treatment.

The invention will be further described with respect to the following examples; however, the scope of the invention is not to be limited thereby;

Example I

General Procedure for Implanting in BNX Mice

The abdomen of an anesthetized mouse was washed with 20 percent ethanol and an incision was made into the abdominal wall to expose the peritoneal cavity. Gelfoam (Upjohn, Kalamazoo, Michigan), cut into strips of approximately 20x15x7mm, or expanded PTFE fibers (W. L. Gore, Inc., Flagstaff, Arizona) pre-coated with collagen IV and pre-weighed (0.2gm dry weight), or a combination of these two materials (Gelfoam strips wrapped with PTFE fibers) were treated with approximately 0.5 ug of HBGF-I in 100 ml of water at 4°C for one hour. Following adsorption of the growth factor the constructed implant device was placed adjacent to the liver of the mouse. The surgical opening was closed by suture or staple gun and the mice were maintained on a normal diet and 12 hour light/dark cycle. At various times post implant (18,28,35 and 38 days) the mice were killed and then

subjected to whole body pressure perfusion with glutaraldehyde, buffered with phosphate, using a catheter inserted into the thoracic aorta. The implants were examined macroscopically for blood vessel formation and the internal organ block removed and thin sections through the entire organ block containing the implant device were prepared for histological examination.

A significant angiogenic response was observed macroscopically and microscopically within 18 days after surgery in all mice (normal (C57) and immune compromised (BNX) mice) treated with HBGFI. Blood vessels, at the tissue site of implantation, were observed macroscopically to be exclusively within the implant. Control implants without HBGF-I did not induce neovascularization. Histological examination of the implants revealed new blood vessel growth within the implants. Total numbers of mice analyzed in this experiment were 3 for C57, and 18 for BNX.

After as long as 40 days, the implants were examined for the extent of angiogenesis. Bidirectional formation of new blood vessels along the HBGF-I implant from the liver and other tissue sites were observed. Further examination revealed that the implant contained vascular "strings" which connected the implant to the existing vascular tree. The ability of HBGF-I implants to maintain these neovessel structures within the peritoneum is evidenced by these highly vascular bridges. Histological examination of these long term implants displayed a large organized solid matrix surrounded by a collagenous capsule (scar) and containing a network of neovessel formations interdigitated with different cell types.. Histological examinations

(longitudinal and cross-section) through typical vascular "strings" revealed the presence of a monolayer of mesothelial cells surrounding a large vascular lumina in the central portion, encompassed by prominent endothelial cells and multiple layers of smooth muscle cells, representing mature and highly differentiated arterioles. Within the periphery are abundant capillary lumina, and the entire vascular bundle is surrounded by a continuous fibrocellular capsule. Further examination of this sample at high magnification revealed the relatively rich collagen component of vascular structure as well as the abundance of endothelial cell-line capillary structures.

In accordance with the device and method of the present invention, angiogenesis and neovascularization has been achieved in the peritoneal cavity of the immune-compromised mouse (BNX).

Example 2

Example 2 demonstrates that the presence of a large vascularized, organized solid matrix, which contains a network of neovessel formations, contiguous with the BNX mouse vascular tree, permits successful transplantation of either a rat-derived hepatoma cell line or a human-derived melanoma cell line into a BNX mouse.

The diploid cell strain, RL-PR-C, a minimally deviated rat hepatoma cell line, and a cell line, A375, derived from a human melanoma, were maintained in culture with either RPMI 1640 (RL-PRC) or high

glucose defined minimal essential media with 10% fetal calf serum, 0.03M glutamine and gentamicin (0.5 mg/ml). These cells were plated on 100mm tissue culture plates and maintained in culture with medium changed every two (2) days for the duration of the experiment.

In order to identify and characterize these tumor cells during the transplantation process, cells were transduced with recombinant retroviral vectors. Either N2 or LNL6 (Armeninano, D., et al., J. Virology 61, 1657-1650; Adam, A.M., and Miller, A.D. (1988) J. Virol. 62, 3802-3806) was used to transfer and integrate the gene for neomycin resistance (Neo®) into the host genomic sequences. Both of the retroviral vectors used in this example are Moloney murine leukemia virus-based vectors with the viral coding sequences removed and the Neo-resistance gene from the bacterial Tn5 transposon inserted. A clone producing a high titer of amphotropic virus containing either N2 or LNL6 was isolated by G418 selection of the helper-free packaging cell line PA317 after calcium phosphate-mediated DNA transfection of the appropriate viral plasmids. Virus-containing supernatants were collected from a confluent monolayer in Dulbecco's minimal essential medium containing 10% fetal calf serum. Following filtration, the viral supernatants were stored at -70°C until used.

Cultured monolayers of either PL-PR-C cells or A375 cells ($2x10^5$) were exposed to virus containing supernatants containing virus particles ($2x10^6$), polybrene (8ug/ml) and appropriate culture medium for 2 hours at 37°C. Following exposure, fresh culture

medium was added and cells were cultured for 48 hours at 37°C. Infected cells, both RL-PR-C (Neo®) and A375 (Neo®) were exposed to G418 (800uq/ml) for two weeks whereupon transduced colonies were expanded for subsequent transplantation.

Transduced tumor cells, RL-PR-C (Neo®) or A375 (Neo®) were grown to high density ($1x10^6$) on Gelfoam sponges (15x12x7mm) and wrapped with 0.2gm of expanded PTFE fibers previously coated with collagen IV and containing adsorbed HBGF-I (0.5ug). This device was surgically implanted in the peritoneal cavity adjacent to the liver of BNX mice as previously described.

At various times post implantation, wrapped sponges were surgically removed and washed extensively in phosphate buffered saline (PBS). Washed implants were digested 30 to 60 minutes at 37°C with a solution of collagenase in PBS (1mg/ml) in a tissue culture incubator (5% CO2). Released cells were collected by centrifugation (10 min, 2000 x g, 20°C) and washed once with PBS and pelleted by centrifugation. Cells were resuspended with appropriate tissue culture medium containing G418 (800ug/ml) and plated for 16 hours on 100mm tissue culture flasks. Plated cells were washed with PBS extensively and fed fresh medium. Medium was changed every two days for the duration of the procedures. Large numbers of viable RL-PR-C (Neo®) cells were recovered at 18, 25 and 34 days post implantation as evidenced by their ability to grow in the presence of G418. This observation was further confirmed by a direct assay of the neomycin phosphotransferase activity (the direct product of the Neo® gene).

Viable A375 (Neo®) cells were recovered at 27, 34 and 40 days post implantation which both grew in the presence of G418 as well as displayed positive neomycin phosphotransferase activity.

Induced neovascularization within the peritoneal cavity has therefore been shown to sustain the proliferative potential of tumor cells simultaneously implanted with HBGF-1 adsorbed solid supports. Tumor cells which were recovered from these HBGF-I implants were able to proliferate in vitro under selective pressure which reflects their genetic disposition.

Example 3

Primary Tumor Cells on Implants in BNX Mice

Primary tumor samples are obtained as biopsy samples from human patients.

These tumor cells, purified from immune system cells if necessary, by standard cell-separation procedures or as pieces of intact tumor, are maintained for a minimal period in vitro in an appropriate medium prior to seeding onto the support for an implant, as described in Examples 1 and 2. The cells on the support are implanted into BNX mice as previously described or as 0.25 to 0.5 cubic centimeter chunks wrapped in 0.1 gm fibers coated with collagen I and 0.25 µg HBGF-1, and a time course of survival of the cells in the implant is carried out. The implant is placed in the peritoneal cavity. Survival and growth of the cells is monitored biochemically, by isolation DNA from cells in the implant, and molecular hybridization with human-specific repetitive sequence DNA probes. Monitoring is also by immunohistochemical means, using implant sections and immune antisera specific

for human and/or tumor cell specific surface markers. Finally, mouse serum can be screened for any human and/or tumor specific secretion products made by cells on the implant and secreted into the circulation.

Having established primary human tumor cells on implants in a series of BNX mice, various therapeutic modalities can be tested to see which are most effective in killing of the patient's tumor cells in vivo in the mouse. The treatments can be but are not limited to regular chemotherapy with established agents, radiation therapy, treatment with biological response modifiers and antitumor agents such as tumor necrosis factor, interleukin 2, α interferon, interferon, interleukin 1, interleukin 4, or a wide range of other such agents.

Response to therapy can be monitored in several ways. Most simple and effective is to monitor a decrease in production and appearance in the mouse's circulation of tumor specific products. Also, the implants can be examined biochemically and histologically to determine the survival of the tumor cells.

Additionally, human bone marrow can be introduced into the BNX mice by published procedures (see references earlier), as a bone marrow transplant, to establish a patient's bone marrow in the immune deficient mouse. Then tumor cells can be implanted, as described above, and a variety of additional cancer therapies, including the adoptive immunotherapy approach, can be tested. These include LAK cell therapy, TIL cell therapy and ALT therapy. Survival of tumor cells is monitored as described above. The adoptive immunotherapy procedures of LAK,

TIL or ALT can be tested with the patients immune cells alone, or in a mouse where the patient's own bone marrow has been reconstituted into the mouse.

Number modifications and variations of this invention are possible; therefore, within the scope of the dependent claims, the invention may be practiced otherwise than as particularly described.

What is Claimed Is:

1. A process for producing an animal model containing human cancer cells comprising: providing a non-human animal with a biocompatable support which includes both biological response modifier and human cancer cells.

2. The process of claim 1 wherein the non-human animal is immunodeficient with respect to its own immune system.

3. The process of claim 2 wherein the biological response modifier is an angiogenic growth factor.

4. The process of claim 3 wherein the growth factor is a heparin binding growth factor.

5. The process of claim 2 wherein the support includes an extracellular matrix component.

6. The process of claim 5 wherein the extracellular matrix component is a collagen.

7. The process of claim 2 wherein the support is a non-absorbable support.

8. The process of claim 7 wherein the support is comprised of polytetrafluoroethylene.

9. The process of claim 2 wherein the non-human animal is a beige/nu/Xid mouse.

10. The process of claim 2 wherein the cancer cells are primary tumor cells.

11. The process of claim 10 wherein the biological response modifier is an angiogenic growth factor and the support further includes an extracellular matrix component.

12. The process of claim 2 wherein the non-human animal includes a human immune system.

13. The process of claim 12 wherein the biological response modifier is an angiogenic growth factor.

14. The process of claim 12 wherein the support includes an extraceullar matrix component.

15. The process of claim 12 wherein the support is a nonabsorbable support.

16. The process of claim 12 wherein the non-human animal is a beige/nu/Xid mouse.

17. The process of claim 12 wherein the cancer cells are primary tumor cells.

18. The process of claim 12 wherein the biological response modifier is an angiogenic growth factor and the support further includes an extracellular matrix component.

19. A process for testing a cancer treatment for human cancer cells, comprising: subjecting a non-human animal to a treatment for human cancer

cells, said non-human animal including cancer cells, said non-human animal being immunodeficient.

20. A process for testing a cancer treatment for human cancer cells, comprising: subjecting a non-human animal to a treatment for human cancer cells, said non-human animal including a biological response modifier and human cancer cells supported on a biocompatable support.

21. The process of claim 20 wherein the non-human animal is immunodeficient with respect to its own immune system.

22. The process of claim 21 wherein the biological response modifier is an angiogenic growth factor.

23. The process of claim 22 wherein the growth factor is a heparin binding growth factor.

24. The process of claim 21 wherein the support includes an extracellular matrix component.

25. The process of claim 21 wherein the support is a nonabsorbable support.

26. The process of claim 21 wherein the non-human animal is a beige/nu/Xid mouse.

27. The process of claim 21 wherein the cancer cells are primary tumor cells.

28. The process of claim 27 wherein biological response modifier is an angiogenic growth factor and the support further includes an extracellular matrix component.

29. A non-human animal model produced by the process of claim 1.

30. The process of claim 2 wherein the support including the cancer cells is implanted in the non-human animal.

31. The process of claim 2 wherein the cancer cells are added to a support implanted in the non-human animal.

32. The process of claim 20 wherein the non-human animal includes a human immune system.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/04344

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 3

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 49/00

U.S.Cl: 424/9

II. FIELDS SEARCHED

Minimum Documentation Searched 4

Classification System	Classification Symbols
U.S.	424/9

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 6

CHEMICAL ABSTRACTS GENERAL SUBJECT INDEX VOL. 96-109 (1982-1988)

"TRANSPLANT"

III. DOCUMENTS CONSIDERED TO BE RELEVANT 14

Category *	Citation of Document, 16 with indication, where appropriate, of the relevant passages 17	Relevant to Claim No. 18
Y	Chemical Abstracts, Volume 99 issued 1983 (Columbus, Ohio, USA) Inoue et al "Antitumor efficacy of seventeen anticancer drugs in human breast cancer xenograft (MS-1) transplanted in nude mice", see page 9, col 2, abstract 98704Z, Cancer Chemother. Pharmacol, 1983, Vol 10(3), 182-6 (Eng).	1,2,9,10,12,16,17 19-21,26,27&29-32
A		3-8,11,13-15,18,22-25& 28
Y	Chemical Abstract, Volume 97 issued 1982 (Columbus, Ohio, USA) Fujita et al, "Characteristic of human gastrointestinal and breast cancer xenografts in nude mice, with special reference to carcinoembryonic antigen and chemosensitivity", page 11, col. 1, abstract 155, 831y, Nippon Geka Gakkai Zasshi, 1982, 83(5), 457-67 (Japan)	1,2,9,10,12,16,17 19-21,26,27&29-32
A		3-8,11,13-15,18,22-25&28

* Special categories of cited documents: 15

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search 2	Date of Mailing of this International Search Report 2
24 October 1990	04 JAN 1991
International Searching Authority 1	**Signature of Authorized Officer 20**
ISA/US	Sam Rosen SamRosen

Form PCT/ISA/210 (second sheet) (May 1986)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category [5]	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
Y A	Chemical Abstracts, Vol 97 issued 1982 (Columbus, Ohio, USA), Warenius et al "In vivo-in vitro clonogenic assays in a human tumor xenograft with high plating efficiency" page 10, col. 2 abstract 84,583f Brit. J. Cancer, 1982, 46(1), 45-50, (Eng)	1,2,9,10,12,16,17, 19-21,26,27&29-32 3-8,11,13-15,18, 22-25&28
Y A	Chemical Abstracts Vol. 98, issued 1983 (Columbus, Ohio, USA), Steel et al. "The response to chemotherapy of a variety of human tumor xenografts" page 7, col. 1 abstract 137,114 h Brit. J. Cancer, 1983, 47(10, 1-13 (Eng)	1,2,9,10,12,16,17, 19-21,26,27&29-32 3-8,11,13-15,18 22-25&28